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Measurement Strategies for the Determination of Airborne Bacterial Endotoxin in Sewage Treatment Plants

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Working in sewage plants can involve exposure to different types of microorganisms, viruses and chemicals. The purpose of this study was to evaluate different measurement strategies to determine airborne bacterial endotoxin in such plants. Sewage treatment plants in three municipalities in western Sweden were included. Measurements of airborne endotoxin were performed in April–May and September–October 2001 using personal and stationary samplers. The air sampling times ranged from 60 to 444 min. In stationary and personal sampler measurements, the amounts of airborne endotoxin detected were generally low. At specific worksites, however, higher endotoxin values were identified, with the highest values at worksites located indoors. The results suggest that the exposure situation is relatively stable over a short time period at a specific worksite and that higher values can be recorded during work practices where agitation of wastewater occurs. The results further suggest that airborne endotoxin exposure situations in sewage treatment plants are complex. Sampling techniques, indoor/outdoor measurements and identification of specific worksites/tasks where there is a risk of airborne endotoxin exposure are important factors that must be considered in order to obtain relevant exposure determinations and establish preventive measures from a health risk perspective.

Keywords: endotoxin, sampling technique, sewage water

INTRODUCTION

There have been several reports of work-related symptoms among employees at sewage treatment plants. They have been summarized in two recent reviews (Mulloy, 2001; Thorn and Kerekes, 2001). Symptoms of eye and nose irritation, lower airway symptoms, fever, fatigue, skin symptoms, headache, dizziness and flu-like symptoms were more common among sewage workers (Mattsby and Rylander, 1978; Clark, 1987; Scarlett-Kranz *et al.*, 1987; Nethercott and Holness, 1988; Zuskin *et al.*, 1993; Melbostadt *et al.*, 1994; Friis *et al.*, 1999; Rylander, 1999). Flu-like symptoms with fever, shivering and headache have been reported among sewage workers handling sludge (Gregersen *et al.*, 1999). Several studies have reported an increased prevalence of symptoms in the gastrointestinal tract (Lundholm and Rylander, 1983; Clark, 1987; Scarlett-Kranz *et al.*, 1987; Friis *et al.*, 1998; Rylander, 1999). An increased risk/prevalence for asthma and chronic bronchitis among sewage workers has also been reported (Zuskin *et al.*, 1993; Friis *et al.*, 1999), as well as decreased lung function values (Nethercott and Holness, 1988; Zuskin *et al.*, 1993; Richardson, 1995).

Working in sewage treatment plants involves exposure to different types of microorganisms, viruses and chemicals. Bacterial exposure is dominated by bacterial species that occur in nature (Lundholm and Rylander, 1983). Most of these bacteria are Gram-negative and endotoxins are thus of particular interest. The acute effects of endotoxins are well documented in several inhalation experiments in humans (Rylander *et al.*, 1989; Herbert *et al.*, 1992; Sandström *et al.*, 1992; Thorn and Rylander, 1998; Michel *et al.*, 2001). Relationships between the amount of

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endotoxin in different environments and respiratory symptoms, spirometry changes and increased inflammatory markers have been reported (Schwartz *et al.*, 1995; Rylander, 1997; Rylander *et al.*, 1999). When handling sewage, endotoxin can be present in amounts that exceed those that give rise to symptoms and illness (Rylander, 1997, 1999; Sarantila *et al.*, 2001), and symptoms among sewage workers have been related to endotoxin exposure (Melbostadt *et al.*, 1994; Rylander, 1999).

The purpose of this study was to evaluate different measurement strategies to determine airborne endotoxin in sewage treatment plants. Questions asked were:

1. Are there specific worksites/practices where exposure to airborne endotoxin occurs?
2. Are stationary and/or personal samplers suitable to determine the relevant airborne exposure in this environment?

MATERIALS AND METHODS

The sewage treatment plants

Sewage treatment plants in three municipalities (Göteborg, Borås and Falkenberg) in western Sweden were included. The sewage plant in Göteborg is the largest plant in Scandinavia, with 55 operatives in daily contact with sewage. The plant has a modern purification process including trickling filters (filters with a nitrification bacterial culture that digests the organic material with nitrate compounds). In Borås and Falkenberg, there were 16 and 14 operatives, respectively. The cleaning procedures for sewage were similar in these two municipalities (aluminium chloride, polyacrylamide and bacterial cultures were used in the purification process).

In Göteborg, airborne endotoxin measurements were performed in April–May 2001. Personal and stationary samplers were used. In Borås, measurements were performed in May and September 2001, also using personal and stationary samplers. In Falkenberg, measurements were performed in October 2001 with stationary samplers. In Borås (in September) and Falkenberg, the air sampling times were 60 min on two different occasions separated by 1 week at each worksite. Double samples using parallel filters were taken at each stationary measurement.

Stationary samplers were placed:

- in different pump stations (a–d);
- in the centrifugation hall (Göteborg);
- at different indoor worksites, including the laboratory, sludge hall, flocculoreaction room and at covered indoor sedimentation basins in two different smaller sewage plants (Borås);
- at different indoor worksites, including the particle grid for incoming water, flocculoreaction room, sludge flotation basin and sludge silo (Falkenberg).

Operatives carried personal samplers:

- during repair work in two pump stations (a and b);
- during inspection and cleaning work in several different pump stations during a workday (Borås);
- in close vicinity to sedimentation basins (Göteborg);
- when working in the sludge separating hall (Göteborg);
- during an ordinary workday (Göteborg);
- in the sludge hall (Borås);
- when handling sludge outdoors (Borås);
- when doing repair work in a sedimentation basin (Borås).

The pump stations were:

(a) Pump station 1, during repair work, with untreated incoming sewage water (Göteborg);
(b) Pump station 2, during repair work, with untreated incoming sewage water (Borås);
(c) Pump station 3, during normal running operations (Borås);
(d) Pump station 4, during normal running operations (Falkenberg).

Exposure determination

Air was sampled by drawing air through Isopore filters (ATTP 0.8 μm; Millipore, Cambridge, MA) using open filter cassettes. For personal samplers, the filters were placed in the breathing zone and connected to a portable pump (Gil-Air 3 SC, Gilian personal air sampler; Gilian Instrument Corp., NJ, USA) with a flow rate of 2 l/min for 100–444 min. The median value was 213 min. The pumps were switched off during the coffee/lunch break. Air samples by stationary samplers were taken at a flow rate of 5 l/min for 60–390 min. The median value was 60 min.

The amounts of airborne endotoxin on the filters were determined by a method previously described (Thorn and Rylander , 1998). In summary, the filters were shaken for 10 min in 10 ml pyrogen-free water. The extracting solution from the filters was analysed for the amount of endotoxin using specific *Limulus* lysate (Tamura *et al.*, 1994). Filter extract samples of 50 μl were placed in a microwell plate and 50 μl of specific endotoxin lysate (Endospecy; Seikagaku Co., Tokyo, Japan) was added. The plate was incubated in a spectrophotometer (Wellreader; Scinics Corp., Tokyo, Japan) and the kinetics of the ensuing colour reaction were read photometrically, transformed into absorbance units and compared to a standard curve. The detection limit for this technique is 10 pg/ml.

The results were expressed in ng/m^3. The conversion factor from nanograms (ng) to endotoxin units (EU) is ~10.

Outdoor and indoor temperatures and relative air humidity were measured on the days of the exposure measurements.

RESULTS

The amounts of endotoxin found in the sewage treatment plants in Göteborg, Borås and Falkenberg are shown in Table 1.

The amounts of airborne endotoxin found with stationary samplers ranged between 0 and 185 ng/m^3. The median endotoxin value was 2.8 ng/m^3. In general, the amounts of airborne endotoxin detected were low, but higher values were found at certain worksites such as the particle grid and the centrifugation hall. The highest values were recorded in the flocculoreaction room in Borås and in pump station 1. In the flocculoreaction room in Falkenberg, lower values were recorded as compared to the same location in the Borås plant.

In the September measurements in Borås (B2) and Falkenberg (F), stationary air sampling was performed on two different occasions separated by 1 week at each worksite. No large differences in recorded values were found between the two measurements. The amounts of airborne endotoxin found in these measurements ranged between 0 and 174.7 ng/m^3 in week 1 and between 0 and 185 ng/m^3 in week 2. The median endotoxin values were 1.1 ng/m^3 in week 1 and 2.2 ng/m^3 in week 2.

The amounts of endotoxin found with personal sampler equipment ranged between 0.1 and 27.2 ng/m^3. The median endotoxin value was 1.3 ng/m^3. Thirteen of the 17 samples were in the range 0.1–2.6 ng/m^3. The highest values were recorded among the workers doing repair work in pump station 1, with untreated incoming sewage water (6.1 and 11.3 ng/m^3), and during repair work in an outdoor sedimentation basin (27.2 ng/m^3). The other observed values were low.

The amounts of airborne endotoxin comparing stationary and personal samplers were by and large similar at worksites/tasks where the sampling techniques were used in parallel (see Table 1).

The outdoor and indoor temperatures during the measurements ranged from 14 to 19°C and 15 to 23°C, respectively, and the relative air humidity ranged from 36 to 86%. No relationships between the measured airborne endotoxin values and outdoor and indoor temperatures and relative air humidity were found (data not shown).

DISCUSSION

The measurements focused on the amounts of airborne endotoxin as a potential candidate for a causative agent for several of the symptoms reported among operatives in previous studies (Rylander, 1999; Thorn and Kerekes, 2001). The results show that the assessment of exposure conditions at a sewage treatment plant is complex. The observed values at any given measuring point must be considered as only rough estimates as they can be influenced by the location of the worksite (indoors/outdoors) and variations in personal exposure situations. In outdoor locations, wind speed and other weather conditions may cause a large variation in measured values at the same site. Differences in measured amounts of endotoxin between different worksites could also be related to local worksite characteristics, particularly the aerosolization of sewage material.

The results demonstrate that specific worksites with higher endotoxin exposures can be identified (pump station 1, the centrifugation hall, the flocculoreaction room in Borås and the particle grid in Falkenberg). Notably, all these worksites were situated indoors. These results suggest that it is possible to identify specific worksites within a sewage treatment plant where there is a risk for endotoxin exposure, especially indoors.

Several previous studies have investigated the risk for endotoxin exposure in sewage treatment plants (Clark, 1987; Melbostadt *et al.*, 1994; Rylander, 1999; Douwes *et al.*, 2001; Mulloy, 2001) and at specific sites such as biofilters and sludge handling (Gregersen *et al.*, 1999; Barth *et al.*, 2002) and in waste treatment plants (Sigsgaard *et al.*, 1990; Douwes *et al.*, 2000). Endotoxin exposure has been suggested as a possible cause of reported symptoms among sewage workers (Mattsby and Rylander, 1978; Lundholm and Rylander, 1983; Rylander, 1999). Laitinen *et al.* (1994) studied the airborne concentrations of endotoxin at nine sewage treatment plants and found the highest endotoxin concentrations in the areas where wastewater or sludge was agitated. This agrees with our results, where higher values were recorded during some work practices where agitation of wastewater occurred (pump station 1 and during repair work in a sedimentation basin). The results of the present study further suggest that specific worksites where there can be a risk for endotoxin exposure can be identified. This agrees with results from previous studies (Rylander, 1999; Sarantila *et al.*, 2001). In our study, the highest endotoxin values were found at worksites located indoors. This agrees with the results of Douwes *et al.* (2001), where significantly elevated endotoxin levels were found at the only worksite located indoors (the sludge dewatering area). The observed values in all other areas were low, but these areas were located outdoors.

Using personal samplers, low amounts of airborne endotoxin were generally found on the filters. The highest values (6.1 and 11.3 ng/m^3) were recorded

Table 1. The amounts of endotoxin found in the sewage treatment plants in Gothenburg, Borås and Falkenberg

Worksite/practice	Stationary samplers					Personal samplers	
	Endotoxin (ng/m^3) week 1		Endotoxin (ng/m^3) week 2		Air sampling time (min)	Endotoxin (ng/m^3)	Air sampling time (min)
	Filter A	Filter B	Filter A	Filter B			
Pump station 1 (during repair work) (G)							
Pump 1/Subject 1	12.5	16.6			280	6.1	177
Subject 2						11.3	223
Pump station 2 (during repair work) (B1)							
Pump 1/Subject 1	1.7[a]				300	2.6	150
Subject 2						1.9	149
Different pump stations (B1)							
Subject 1						0.5	267
Subject 2						5.2	100
Pump station 3 (B2)							
Pump 1	0	0	0.2	0	60		
Pump station 4 (F)							
Pump 1	0	0	0	0	60		
Centrifugation hall (G)							
Pump 1	5.7	13.8			260		
Sludge hall (B1)							
Pump 1/Subject 1	0.5	0.5			390	1.5	260
Subject 2						1.2	212
Pump 2 (B2)	1.2	0.2	2.2	0.8	60		
Laboratory (B2)	0.4	0.9	0.6	0.5	60		
Flocculoreaction room (B2)	115.6	174.7	185.0	168.2	60		
Indoor sedimentation basin 1 (B2)	12.6	2.3	6.2	2.9	60		
Indoor sedimentation basin 2 (B2)	3.9	1.4	2.1	3.3	60		
Particle grid for incoming water (F)	7.1	6.9	12.6	15.9	60		
Flocculoreaction room (F)	5.8	7.4	4.3	2.2	60		
Sludge silo (F)	0.3	0.4	2.2	0.9	60		
Sludge flotation basin (F)	0.2	0.2	0.6	0.5	60		
Sedimentation basins (G)							
Subject 1						0.2	444
Subject 2						1.3	327
Sludge separating hall (G)							
Subject 1						1.0	353
Ordinary work practices (G)							
Subject 1						0.6	132
Subject 2						0.9	416
Subject 3						0.3	348
Subject 4						1.4	189
Handling sludge outdoors (B1)							
Subject 1						0.1	213
Repair work in a sedimentation basin (B1)							
Subject 1						27.2	102

One filter was analyzed at each measurement using personal samplers and parallel filters were analyzed using stationary samplers.
B1, May measurements in Borås; B2, September measurements in Borås; F, Falkenberg; G, Göteborg.
Week 1/week 2, all measurements in Borås (in September) and Falkenberg were performed on two different occasions separated by 1 week at each worksite.
[a]Only one filter was analyzed as the other was lost during the technical procedure.

during repair work in pump station 1 with untreated incoming sewage water and during repair work on an outdoor sedimentation basin (27.2 ng/m^3). The values found in pump station 1 were largely in correspondence with the values found with stationary samplers (see Table 1). This agrees with the low corresponding endotoxin values found when using stationary and personal samplers during repair work in pump station 2. This suggests that either stationary or personal sampler equipment can be used to determine the exposure during a specific work task. This is, however, based on a limited number of measurements when a specific work task was performed. The initial effort was to perform a more extensive comparison between personal and stationary samples at the specific worksites but this was not possible from a practical point of view, as the operatives moved between sites all over the plant during an ordinary workday. In summary, stationary samplers are often the only alternative to determine the specific worksite exposure, but personal samplers are preferable to determine the overall exposure for an individual operative during an ordinary workday.

From a health–exposure point of view it is important to determine the exposure during specific work tasks as a short peak exposure could induce effects. Air sampling time can be an important factor. In this study the sampling times were 60–444 min. In the studies by Rylander (1999) and Sarantila *et al.* (2001) air was sampled for 60 min. In the study by Douwes *et al.* (2001) the air sampling times were 8 h up to 4 days, which may increase the risk that relevant peak exposures were diluted.

To determine exposures, repeated measurements are usually recommended. In this study, however, no large differences in recorded values were found between the two measurements performed 1 week apart in Borås and Falkenberg, suggesting a relatively stable exposure situation over a short time period at a specific worksite in sewage treatment plants. In general, no large differences were found in endotoxin values between parallel filters taken at the same time, indicating a rather homogeneous exposure situation during the sampling time. In the sludge hall in Borås, where measurements were performed in May and September, similar endotoxin values were found.

At certain worksites the measured endotoxin values exceeded the suggested guidelines for airborne endotoxin exposure in the environment, i.e. the no-effect level for airways inflammation (10 ng/m^3) during an 8 h workday (Rylander, 1997). The Dutch Health Council has proposed a health-based exposure standard of 50 EU/m^3 for endotoxin exposure. This has been adapted to a higher value of 200 EU/m^3, which will become the standard in The Netherlands from July 2003 (D. Heederik, personal communication). Mulloy (2001) has recently suggested that permissible endotoxin levels established in other environments should be adopted for the wastewater environment.

In conclusion, the results suggest that airborne endotoxin exposure in sewage treatment plants is complex. The main findings were that specific worksites where there can be a risk for endotoxin exposure can be identified and the highest endotoxin values were found at worksites located indoors. The results suggest that the exposure situation is relatively stable over a short time period at a specific worksite and that higher values can be recorded during work practices where agitation of wastewater occurs. Sampling techniques, indoor/outdoor measurements and identification of specific worksites/tasks where there is a risk of airborne endotoxin exposure are important factors that must be considered in order to obtain relevant exposure determinations and establish preventive measures from a health risk perspective.

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